

U.S.S.N. 09/731,632  
Case No. 18906IAR  
Page No. 2

This listing of claims will replace all prior versions and listings of claims in the application:

**Listing of Claims:**

Claim 1. (Previously amended) An assay for determining the cyclooxygenase-2 activity [of a sample] of a composition comprising a human osteosarcoma cell preparation, a sample and arachidonic acid, the assay comprising the steps of:

(a) preparing the composition by [adding]

(1) [a] the human osteosarcoma cell preparation,

(2) [a] the sample, said sample comprising a putative cyclooxygenase-2 inhibitor,  
and

(3) the arachidonic acid; and

(b) determining the amount of prostaglandin E<sub>2</sub> produced in step (a).

Claim 2. (previously amended) An assay [for determining the cyclooxygenase-2 activity of a sample] according to claim 1 [comprising the steps of:

(a) adding

(1) a human osteosarcoma cell preparation,

(2) a sample, said sample comprising a putative cyclooxygenase-2 inhibitor, and

(3) arachidonic acid; and

(b) determining the amount of prostaglandin E<sub>2</sub> produced in step (a). ]

wherein the cell preparation comprises 10<sup>3</sup> to 10<sup>9</sup> whole cells of osteosarcoma per cc, or 50 to 500 ug of osteosarcoma microsomes per ml of preparation; and 0.1 to 50 µl of arachidonic acid per ml of preparation.

U.S.S.N. 09/731,632  
Case No. 18906IAR  
Page No. 3

Claim 3. (Previously amended) An assay for determining the cyclooxygenase-2 activity of [a sample] a composition comprising a human osteosarcoma cell preparation, a sample and arachidonic acid, the assay comprising the steps of:

(a) preparing the composition by [adding]

- (1) [a] the human osteosarcoma cell preparation,
- (2) [a] the sample, said sample comprising a putative cyclooxygenase-2 inhibitor, and
- (3) the arachidonic acid; and

(b) determining the amount of prostaglandin E<sub>2</sub> produced in step (a)

(c) correlating the amount of prostaglandin E<sub>2</sub> produced with cyclooxygenase-2 activity, wherein the osteosarcoma cell preparation consists essentially of osteosarcoma 143.98.2 microsomes.

Claim 4. (Original) An assay according to claim 3 wherein the osteosarcoma 143.98.2 microsomes are substantially free of endogenous arachidonic acid.

Claim 5. (Original) An assay according to claim 3 wherein the microsomes are contacted with an amount of delipidized serum protein effective to reduce the amount of endogenous arachidonic acid in the microsomes by a factor of at least approximately 2.

Claim 6. (Previously amended) An assay for determining the cyclooxygenase-2 activity of [a sample] a composition comprising a human osteosarcoma cell preparation, a sample and arachidonic acid, the assay comprising the steps of:

(a) preparing the composition by [adding]

- (1) [a] the human osteosarcoma cell preparation,

U.S.S.N. 09/731,632  
Case No. 18906IAR  
Page No. 4

(2) [a] the sample, said sample comprising a putative cyclooxygenase-2 inhibitor,  
and

(3) the arachidonic acid; and

(b) determining the amount of prostaglandin E<sub>2</sub> produced in step (a),

(c) correlating the amount of prostaglandin E<sub>2</sub> produced with cyclooxygenase-2 activity,

wherein the human osteosarcoma cell preparation contains no recombinant vector.

Claim 7. (Previously amended) An assay for determining the cyclooxygenase-2 activity of [a sample] a composition comprising a human osteosarcoma cell preparation, a sample and arachidonic acid, the assay comprising the steps of:

(a) preparing the composition by [adding]

(1) [a] the human osteosarcoma cell preparation,

(2) [a] the sample, said sample comprising a putative cyclooxygenase-2 inhibitor,  
and

(3) the arachidonic acid; and

(b) determining the amount of prostaglandin E<sub>2</sub> produced in step (a)

(c) correlating the amount of prostaglandin E<sub>2</sub> produced with cyclooxygenase-2 activity,

wherein the osteosarcoma cell preparation consists essentially of whole cells of osteosarcoma 143.98.2.

Claim 8. (Canceled).

Claim 9. (Previously amended) A composition [according to claim 8] comprising:

U.S.S.N. 09/731,632  
Case No. 18906IAR  
Page No. 5

(a) an osteosarcoma cell preparation having  $8 \times 10^4$  to  $2 \times 10^6$  osteosarcoma 143.98.2 whole cells per cc of cell preparation or 100 to 400 µg of osteosarcoma 143.98.2 microsomes; and 10 to 20 µl of peroxide-free arachidonic acid per cc of cell preparation; and

(b) 0.1 to 50 µl of [10 to 20 µl of peroxide free] arachidonic acid per cc of cell preparation.

Claim 10. (Original) A composition according to claim 9 wherein the microsomes are substantially free of endogenous arachidonic acid.

Claim 11. (Previously amended) An assay for determining the cyclooxygenase-1 activity of [a sample] a composition comprising the steps of:

(a) preparing the composition [adding]

(1) [a] the COX-1 cell preparation,

(2) [a] the sample, said sample comprising a putative cyclooxygenase-1 inhibitor;

(3) the arachidonic acid; and

(b) determining the amount of prostaglandin E<sub>2</sub> produced in step (a)

(c) correlating the amount of prostaglandin E<sub>2</sub> produced with cyclooxygenase-2 activity.

Claim 12. (Original) An assay according to claim 11 wherein the COX-1 cell preparation consists essentially of whole cells of U-937.

Claim 13. (Original) An assay according to claim 11 wherein the COX-1 cell preparation consists essentially of U-937 microsomes.

Claim 14. (Previously amended) An assay according to claim 11 [for determining the cyclooxygenase-1 activity of a sample comprising the steps of:

(a) adding

U.S.S.N. 09/731,632  
Case No. 18906IAR  
Page No. 6

- (1) a COX-1 cell preparation,
- (2) a sample, said sample comprising a putative cyclooxygenase-1 inhibitor;
- (3) arachidonic acid; and

(b) determining the amount of prostaglandin E<sub>2</sub> produced in step (a),]

wherein the cell preparation comprises 10<sup>5</sup> to 10<sup>8</sup> whole cells of U-937 per cc, or 1 to 10 mg of U-937 microsomes per ml of preparation; and

0.1 to 50 µl of arachidonic acid per ml of preparation.

Claim 15. (Original) An assay according to claim 14 wherein the cell preparation comprises 8x10<sup>8</sup> to 1.5x10<sup>6</sup> whole cells of U-937 per cc, or 1 to 5 mg of U-937 microsomes per ml of preparation.

Claims 16 to 18 (Canceled)

Claim 19. (Previously amended) A transformed host cell that expresses cyclooxygenase-2 as shown in SEQ. ID. NO: 10 [FIG. 1 (SEQ. ID. NO. 10:)]comprising:

- (a) a mammalian or eukaryotic expression vector; and
- (b) a sequence encoding human cyclooxygenase-2 comprising bases 97 to 1909 of [as shown in FIG. 2 (JSEQ. ID. NO: 11)] or encodes protein of [FIG. 1 (JSEQ. ID. NO: 10)].

Claims 20 to 21 (Canceled).

Claim 22. (Previously amended) Human cyclooxygenase-2 cDNA comprising the coding region which is bases 97 to 1909 of SEQ. ID. NO: 11.

Claim 23. (Currently amended) Recombinant human cyclooxygenase-2 [which is shown in] consisting of SEQ. ID. NO: 10.

U.S.S.N. 09/731,632  
Case No. 18906IAR  
Page No. 7

Claim 24. (Currently amended) An isolated human cyclooxygenase-2 [which is shown in] consisting of SEQ. ID. NO: 10.

Claim 25. (Currently amended) Purified human cyclooxygenase-2 [which is shown in] SEQ. ID. NO: 10.

Claim 26. (Previously amended) The transformed host cell according to claim 19 wherein the expression vector is a vaccinia or baculovirus vector.

Claim 27. (Previously amended) The transformed host cell according to claim 19 wherein the cyclooxygenase-2 is expressed in COS-7 cells.